Annex D

Joint Medical Chemical, Biological, and Nuclear Defense Research Programs

The joint medical chemical, biological, and nuclear (radiological) defense research programs are each addressed in the next three sections.

D.1 MEDICAL CHEMICAL DEFENSE RESEARCH PROGRAM

D.1.1 Fielded Products

Advances in medical research and development (R&D) significantly improve the warfighting mission by sustaining unit effectiveness through conserving the fighting strength of our forces and supporting the nation's global military strategy, which requires the ability to effectively deploy and operate. Medical R&D products (materiel and non-materiel solutions) provide the foundation that ensures the fielding of a flexible, sustainable, modernized force across the spectrum of conflict and in the full breadth and depth of the battlefield. Overcoming medical threats and extending human performance have provided a significant increase in military effectiveness in the past and present the potential for future enhancement of military operational effectiveness. Some fielded medical chemical defense R&D materiel and non-materiel solutions are:

Pharmaceuticals:

- Nerve Agent Antidote Kit (Mark I), 1983
- Skin Decontamination Kit (M291), 1990
- Nerve Agent Pretreatment (Pyridostigmine), 1985
- Convulsant Antidote for Nerve Agent (CANA), 1991
- Medical Aerosolized Nerve Agent Antidote (MANAA), 1994

MARK I, M291, Nerve Agent Pretreatment, and CANA

MANAA

Materiel:

- Test Mate® ChE (Cholinesterase) Kit, 1997 (*shown*)
- Resuscitation Device, Individual, Chemical, 1990
- Decontaminable Patient Litter (NSN 6530-01-380-7309), 1991
- Chemical Warfare (CW) Protective Patient Wrap (NSN 8415-01-311-7711), 1991
- Computer-Based Performance Assessment Battery, 1993
- M40 Protective Mask Vision Correction (optical inserts)

Decontaminable Patient Litter and CW Protective Patient Wrap

Technical Information and Guidance:

- Taxonomic Work Station, 1985
- U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) Technical Memoranda on Chemical Casualty Care, 1990
- Field Manual (FM) 8-285, "Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries," 1990
- Handbook, "Medical Management of Chemical Casualties," 1995
- Field Management Handbook, "Medical Management of Chemical Casualties," 1996
- Technical Bulletin (TB) Medical (MED) 296, 1996
- Compact Disk - Read-Only Memory (CD-ROM) on "Management of Chemical Warfare Injuries," 1996

D.1.2 Medical Chemical Defense R&D Accomplishments

The medical chemical defense R&D technical barriers and accomplishments during FY98 are grouped by medical chemical defense strategies, which include the following:

- Prophylaxes
- Pretreatment
- Therapeutics
- Diagnostics

Today's chemical threat, however, is not restricted to commonly accepted classical agents, such as vesicants [sulfur mustard (HD)], nerve agents (soman, sarin, tabun, and VX), respiratory agents (phosgene), or blood agents (cyanide). Potential adversaries may develop novel threat agents. The ability to provide timely and effective medical countermeasures to new threats depends upon maintaining a high level of technological capability.

Countermeasure strategies to the classic and novel threats include pharmaceuticals, medical equipment, specialized materiel or medical procedures, and concepts for training,

doctrine, and organization. Medical countermeasures are designed not only to prevent lethality but also to preserve and sustain combat effectiveness in the face of combined threats from chemical and conventional munitions on the integrated battlefield by:

- Prevention of the effects of chemical agents (*e.g.*, prophylaxes or pretreatment).
- Far-forward treatment upon exposure to chemical warfare threats (*e.g.*, antidotes).
- Chemical casualty care (*e.g.*, therapy and management).
- Rapid diagnosis of chemical agent exposure.

Research Category: Prophylaxes/Pretreatments

The countermeasures, technical barriers, and accomplishments in the medical chemical defense research category of prophylaxes/pretreatments are outlined below.

Countermeasures:

- Reactive topical skin protectant (rTSP) for chemical agents.
- Pretreatment regimen that protects against rapid action and incapacitating effect of chemical threat category of nerve agents and novel threat agents.
- Pharmaceutical/biological pretreatments, treatments, antidotes or decontaminants/protectants.

Technical Barriers:

- Lack of appropriate model systems for testing treatment efficacy and safety in humans.
- Lack of pretreatments/antidotes that are quick acting, long lasting, easy to carry and use on the battlefield.
- Lack of appropriate experimental model systems to predict pretreatment or treatment efficacy and safety in humans.
- Lack of detailed molecular model of novel threat agents to understand the origin of their unique chemical properties.
- Potential performance decrement with pretreatment under investigation unless effects are closely monitored during administration.

Accomplishments:

- Observed that neonatal mice fail to develop HD lesions comparable to those seen in weanling mice.
- Identified a prototype formulation for rTSP that dramatically increases protection against HD vapor.
- Developed a method for preparing crystals of *T. californica* acetylcholinesterase (AChE) inhibited with sarin, soman or diisopropylfluorophosphate. Refinement of three-dimensional structure is almost complete (collaboration with the Weizmann Institute, Israel).
- Examined inhibition rates of butyrylcholinesterase (BuChE) mutants having organophosphorus (OP) anhydrolase activity by carbamates and determined that slow reactivity of BuChE mutants with OP probably results from interference of transition state stabilization by the bulky histidine sidechain that was introduced.

- Elucidated control mechanism of *in vitro* secretion of carboxylesterase (CaE) by mutagenesis of C-terminal of CaE.
- Expressed human CaE for use as an exogenous scavenger for OP agents.
- Made four double mutants of CaE each with altered C terminal residues; of these, two had a histidine introduced near the active site and two had a glutamine introduced near the active site.
- Observed that cholinesterase (ChE) attached to a solid support has enhanced stability over soluble forms of the enzyme.
- Found that differences in terminal elimination rates of soman in guinea pig and marmoset *vs*. rat correlated with differences in the levels of soman binding sites in liver of these species.
- Found that tissue/plasma partition coefficients of soman in rat, guinea pig, and marmoset were essentially equal suggesting that the pharmacokinetic distribution of soman in these species should be quite similar (collaboration with Prins Maurits Laboratory, TNO).
- Standardized the Chinese hamster ovary expression system for BuChE and CaE expression.
- Developed a more sensitive and safe method to determine partition coefficients of nerve agents.
- Found differences in the oligosaccharides of native and recombinant CaEs with regard to the total carbohydrate content and charge- and size-based oligosaccharide profiles.
- Determined that neither the carbohydrate composition nor the oligosaccharide profile could be completely correlated with the pharmacokinetic parameters of these enzymes.
- Explored synthesis of a monoclonal anti-soman antibody for further development as a ‘dip-stick’ diagnostic product (collaboration with Army Research Laboratory).
- Created a database for physiologically based pharmacokinetic (PB/PK) parameters for rat, guinea pig, monkey, and human; these parameters are being evaluated for allometric consistency to develop a simplified PB/PK model that can predict nerve agent toxicokinetics regardless of species (collaboration with Prins Maurits, TNO).
- Developed a PB/PK computer model for inhalation exposure to soman (collaboration with Prins Maurits, TNO).
- Determined percutaneous median lethal doses of five novel threat agents in guinea pigs.
- Measured the rates of absorption of three novel threat agents administered by subcutaneous and percutaneous routes in guinea pigs.
- Evaluated the distribution of three novel threat agents in rodents after subcutaneous administration.
- Measured the physiological effects of five novel threat agents on electrocorticographic, respiratory, electromyographic, and cardiovascular parameters in guinea pigs.
- Demonstrated that the mechanism of toxicity of novel threat agents was due to their inhibition of AChE and the resulting elevation of acetylcholine (ACh) levels in the nervous system.
- Physicochemical measurements revealed that novel threat agents were not ionized under physiological conditions and were hydrolyzed at a slower rate than conventional nerve agents.
- Demonstrated that carbamate pretreatment was required for significant protection by current medical countermeasures against three of the novel threat agents.

- Elucidated the structural/functional relationship between the glycosylation and the pharmacokinetic behavior of ChEs. Successful application of native and recombinant ChEs as detoxifying drugs largely depends on their ability to remain at therapeutic plasma levels for prolonged periods. Variations in ChE charge, structure, and oligosaccharide content are factors in establishing ChE mean residence time *in vivo*.
- Demonstrated that serum- and tissue-derived AChEs are more effective bioscavangers than recombinant DNA-derived AChEs as potential candidates for pre- or postexposure treatment for OP toxicity.
- Demonstrated that reinhibition of organophosphate-inhibited AChE by phosphoryl oxime depends on the structure of the oxime reactivator and the organophosphate used.
- Established that in simultaneous acute exposure to DEET, permethrin, and pyridostigmine, there was no synergistic inhibition of binding to muscarinic or nicotinic receptors or inhibition of cholinestrase activity.
- Demonstrated differences in the active-site gorge dimensions of AChEs and BuChEs using data gathered from inhibition studies with BuChE.
- Elucidated the complete amino acid sequence of equine serum BuChE, a protein of 574 amino acids.
- Showed that monoclonal antibodies that inhibit catalytic activity of AChE do so, in part, by allosterically affecting the orientation of tryptophane 86, located at the base of the active-site gorge.

Research Category: Therapeutics/Diagnostics

The countermeasures, technical barriers, and accomplishments in the medical chemical defense research category of therapeutics/diagnostics are outlined below.

Countermeasures:

- Products that prevent or moderate vesicant injury.
- Medical countermeasures to minimize lethality, morbidity, and incapacitation of these agents.
- Specific casualty management techniques to improve survival and minimize lost duty time.
- Pharmaceutical/biological pretreatments, treatments, antidotes, or decontaminants/ protectants.

Technical Barriers:

- Need for quick-acting and long-lasting antidotes that are deployable.
- Lack of appropriate experimental model systems for treatment efficacy and safety in humans.
- Need for detailed molecular model of novel threat agents to understand the origin of their unique chemical properties.

Accomplishments:

- Determined that the cytokines IL-1b, IL-6, TNF-alpha, and MIP-1a mRNA levels are dramatically increased following cutaneous HD exposure in the mouse ear.

- Showed that two precursor enzymes for substance P are elevated following cutaneous HD exposure in the mouse ear.
- Found a weak but positive signal for the presence of NFkB, an inflammatory response regulator, in lung tissue within 6 hours after HD exposure.
- Observed that inhalation exposure to HD in rats results in a significant leukocyte suppression at 24 hours after the exposure.
- Developed a mathematical model of anaerobic glycolysis that was used to test the hypothesis that HD-induced inhibition of glycolysis is mediated by NAD+ depletion.
- Using a monoclonal antibody against DNA ligase I, affinity column chromatography confirmed that activation of DNA ligase following HD exposure is through phosphorylation.
- Showed upregulation of 100 gene transcripts including multiple inflammatory protein transcripts such as intracellular adhesion to molecule-1 and interleukin-8 following HD exposure.
- Modified the single cell comet assay for DNA strand breakage for detection of the effects of HD crosslinking on the demonstration of strand breaks caused by H_2O_2.
- Demonstrated that exposure of keratinocytes to HD leads to cytotoxicity involving terminal differentiation and apoptosis via a calcium-calmodulin and caspase-dependent pathway.
- Assessed the toxicokinetics of HD in the hairless guinea pig following IV administration of 0.3 LD_{50} using gas chromatography coupled with PFPD and showed that the half-lives of distribution and elimination were 0.7 and 152 minutes, respectively.
- Found 17 candidate medical countermeasures that provide significant reduction in HD-induced edema, histopathology, or both in the mouse ear assay.
- Determined that 6 of the compounds showing a statistical reduction of injury in the mouse ear assay produced greater than 50% reduction of edema or histopathology.
- Measured the ability of oximes to reactivate enzymes inhibited by novel threat agents and correlated the refractoriness of novel threat agents to medical countermeasures with the inability of oximes to reactivate novel agent-inhibited AChE.
- Established nonhuman primate electroencephalographic (EEG) recording model to assess anticonvulsant action of current treatment (diazepam) *vs.* proposed new anticonvulsant therapies for nerve agent-induced seizures.
- Determined that the benzodiazepine, midazolam, provides more rapid and more potent anticonvulsant action against nerve agent-induced seizures than the current therapy diazepam.
- Established that certain anticholinergic drugs in combination with benzodiazepines provide more potent anticonvulsant action against nerve agent seizures than either class of drug by itself.
- Determined that two neuroactive steroids with purported anticonvulsant activity could neither prevent nor stop nerve agent seizures.
- Demonstrated that the anticholinergic drug biperiden provides potent anticonvulsant activity against all nerve agents to include the novel threat compounds.
- Established that the drug baclofen, a compound that acts preferentially at GABA-B receptor sites, is not effective as an anticonvulsant against nerve agent-induced seizures.
- Identified compounds that can act as neuroprotectant agents against brain damage pro-

duced by nerve agent seizures. These compounds may act by preventing destabilization of calcium homeostasis, or as free radical scavengers, or both. Some are able to prevent the seizure-induced damage without influencing the severity or duration of seizure activity. Such compounds could be used in addition to traditional anticonvulsant drugs to protect severely poisoned casualties against the neurotoxic effects of nerve agent exposure.

- Determined that calcium channel blockers such as nifedipine do not increase survival rates of mice exposed to phosgene.
- Developed a mouse model that allows for the determination of arterial blood gas and electrolyte status over 24 hours in mice after exposure to phosgene.
- Determined that there are increases in blood potassium, hematocrit, hemoglobin, ionized calcium, and sodium in mice exposed to phosgene.
- Developed a porcine model to investigate the use of positive end expiratory pressure in the treatment of phosgene exposure.
- Found that 6 hours after exposure of lung tissue to HD, there was a dose-response change in the concentration of protein, an early marker of acute lung injury in the bronchoalveolar lavage.
- An antisense oligodeoxynucleotide construct based on amino acid sequence of HD-stimulated protease prevents protease mRNA expression induced by HD.
- Performed rat EEG studies and determined that the muscular tremor and high-dose lethal effects of huperzine, a potential nerve agent antidote and anticonvulsant, are not associated with cortical brain seizure activity.
- Evaluated the possible utility of kainic acid-induced sustained cortical EEG seizures and status epilepticus as a preclinical rat model mimicking agent-like, antiepileptic drug-resistant brain seizures.
- A study was undertaken to determine if abnormally low blood ChE activity, abnormal red blood cell acetylcholinesterase (RBC-AChE), PB inhibition kinetics, and/or unusually high frequencies of the atypical phenotype of plasma BuChE could explain some of the symptoms exhibited by Gulf War veterans or represent a risk factor for adverse effects after PB exposure. Sampling of Gulf War veterans showed no evidence of unusually low ChE activity or altered RBC-AChE kinetics, as evaluated by determination of spontaneous reactivation time after PB inhibition.
- Developed a prototype, noninvasive finger-cuff optical probe to simultaneously monitor continuous measurements of oxyhemoglobin, deoxyhemoglobin, methemoglobin and carboxyhemoglobin for use in cyanide exposure.
- Demonstrated that ChE 'sponges' could neutralize nerve agents and then be reused up to five times after oxime regeneration with only a 30% loss of initial activity.
- Established a laboratory for 24-hour EEG monitoring for cholinergically induced seizures in freely moving rats, to permit high-throughput screening for novel anticonvulsants.
- Developed noninvasive technique (Dynamic Area Telethermometry) to evaluate mustard and other exposures (*i.e.*, nerve gas) to the skin.
- Conducted experiments to calibrate and verify noninvasive optical probe monitor used to monitor pretreatment decrements. Preliminary analysis showed efficacy to be comparable to the oximeter instrument (OSM3) currently employed.

- Showed that postexposure therapy with bioscavenger ChE was effective against residual anticholinesterase activity produced by chlorpyrifos exposure as much as four hours earlier.
- Developed a product composed of ChEs, oxime, and polyurethane foam for removal and decontamination of OP compounds from biological surfaces such as skin that also can be used to develop methods for safe disposal of stored OP nerve agents.
- Characterized the interaction of anti-Alzheimer drugs, huperzine A and E2020 (Aricept®), with ChEs, showing that both inhibitors display a high level of selectivity for AChE over BuChE and that major interactions are with aromatic residue Tyrosine 337 in the active-site gorge of AChE.
- Concluded that huperzine A may interfere with and be beneficial for excitatory amino acid overstimulation, which has been postulated to cause neuronal cell death.
- Showed that stable complexes formed by AChE and amyloid-β-Peptide may increase the neurotoxicity of Aβ fibrils and thus may determine the selective neuronal loss observed in Alzheimer's brain.
- Found that a monoclonal antibody directed against fetal bovine serum AChE inhibited promotion of Alzheimer amyloid fibril formation triggered by AChE (collaboration with Pontificia Universidad Católica de Chile).

D.1.3 Advanced Development Products

In advanced development, the goal is proof-of-principle and conducting all studies necessary to obtain FDA approval/licensure of drugs, vaccines, and devices. The medical R&D process links the materiel developer (U.S. Army Medical Research and Materiel Command [USAMRMC]) with the combat and training developer (Army Medical Department Center and School [AMEDD C&S]) and the logistician in addressing the threat and Department of Defense (DoD) requirements. Medical chemical defense products now in the advanced development phase are the following:

Product: Topical Skin Protectant (TSP)

Concept:

- Use perfluorinated formulations.
- Form nontoxic, nonirritating barrier film layer on skin.
- Augments Mission Oriented Protective Posture (MOPP).
- Protection against vesicant and nerve agents.

Status:

- Two candidates transitioned to demonstration/validation phase.
- Candidates demonstrated efficacy against broad spectrum of threat agents; down-selected to one candidate.
- Investigational New Drug (IND) application submitted to the FDA.
- Demonstrated the human safety and technical performance of the TSP.
- Demonstrated extended stability of the TSP.
- Validated production/manufacturing capability for the TSP.

- Awarded a manufacturing development contract.
- NDA is under preparation.

Product: Multichambered Autoinjector

Concept:

- Speed administration of life-saving antidotes against nerve agents.
- Replace two-Injector Mark I Nerve Agent Antidote Kit with single autoinjector.

Status:

- Engineering contract awarded in September 1993.
- Fielding will require full FDA approval.
- Demonstrated the human safety of the multi-chambered autoinjector.
- Engineering and development of final prototype completed.

Product: Cyanide Pretreatment

Concept:

- Provide protection against incapacitation and lethality without performance degradation.
- Enhance soldier protection and sustainment.

Status:

- Completed preclinical toxicology and drug distribution studies.
- Developed dose parameters and performance assessments.
- Concluded animal toxicology studies for cyanide pretreatment.
- Completed preparation of IND application.
- Initial efforts to conduct first human safety tests.
- Draft Engineering and Manufacturing Development Request for Proposals undergoing staffing.

D.2 MEDICAL BIOLOGICAL DEFENSE RESEARCH PROGRAM

D.2.1 Biological Defense Products

Advances in DoD medical R&D significantly impact the warfighting mission by sustaining unit effectiveness through conserving the fighting strength of our soldiers and supporting the nation's global military strategy, which requires the ability to effectively deploy and operate. Medical R&D products (materiel and non-materiel solutions) provide the foundation that ensures the fielding of a flexible, sustainable, modernized force across the spectrum of conflict and in the full breadth and depth of the battlefield. Overcoming medical threats and extending human performance have provided a significant increase in military effectiveness in the past and present the potential for future enhancement of military operational effectiveness. Some of the materiel and non-materiel solutions developed for use in medical biological defense R&D include the following:

Vaccines and Antisera:

- Anthrax Vaccine (licensed)
- Plague Vaccine (licensed)*
- Smallpox Vaccine (licensed)
- Botulinum Toxoid Vaccine, Pentavalent (IND #3723)
- Botulinum Type F Toxoid Vaccine (IND #5077)
- Botulinum Antitoxin, Heptavalent Equine (Types A, B, C, D, E, F, and G) (IND #3703)
- Botulism Immune Globulin, Human (IND #1332)
- Botulism, Antitoxin, Heptavalent Equine, Types A, B, C, D, E, F, and G (IND #5077)
- Q Fever Vaccine, Purified Whole Cell, CM Residue, Formalin Inactivated, Gamma Irradiated (IND #3516)
- Tularemia Vaccine (IND #157)
- Vaccinia Virus Vaccine, Cell Cultured (IND #4984)
- Venezuelan Equine Encephalitis Virus Vaccine, TC-83 (IND #142)
- Eastern Equine Encephalitis Virus Vaccine (IND #266)
- Western Equine Encephalitis Virus Vaccine (IND #2013)

**Plague vaccine is licensed against bubonic plague but is probably not effective against aerosolized* Yersinia pestis *(Plague)*

Technical Information and Guidance:

- Handbook "Medical Management of Biological Casualties," 1998.
- In FY98, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), in collaboration with the Centers for Disease Control and Prevention, broadcast a live, interactive satellite distance learning course entitled "Medical Response to Biological Warfare and Terrorism" to 17,319 military and civilian health professionals and first responders at 500 sites across the United States. This 3-day course proved to be very cost-effective, as the cost was $69 per student trained; whereas, it costs an estimated $1,000 to train a health care provider at USAMRIID's resident in-house course, which is

given four times yearly to 76 students per course. This satellite distance learning course represented a new era in cooperation with a civilian government agency to provide important information to all who may confront threats from biological agents.
- CD-ROM on "Management of Biological Warfare Casualties" late fall 1999.

D.2.2 Biological Defense Research and Development Accomplishments

The biological defense research and development technical barriers and accomplishments during FY98 are grouped by biological threat category, which include the following:

- Bacterial (and rickettsial) agents.
- Protein toxins.
- Viral agents.

In addition, research and development accomplishments in the area of confirmatory diagnostic assays for biological warfare threat agents are presented at the end. The objective of this effort is to sustain and enhance the capability to confirm in biological samples the initial field diagnosis/identity of a biological warfare threat agent indicated by initial field screening.

Several projects and technologies are shared with other agencies, including the Department of Energy (DOE) and the Defense Advanced Research Projects Agency (DARPA). The DOE projects tie into the strengths of the DOE laboratories in developing advanced technologies in order to enable rapid detection of and response to a chemical or biological agent incident. DOE is not involved directly in protection and treatment of personnel, but actively assists DoD with drug/chemical database searches, DNA sequencing, advanced protein chemistry and modeling/simulation projects. Successful sequencing of plasmids found in the causative agents of plague and anthrax helped create the "lab on a chip" that is a hand-held chromatography laboratory. The extensive knowledge and databases available to DOE allow application of computational tools to predict sites of intervention by novel therapies against threat agents.

DARPA is pursuing multi-agent and broad-spectrum approaches, both to defend against current known threats and to anticipate potential future threats. Accomplishments of DARPA programs for FY98 include the following:

Medical Countermeasures Research and Development by DARPA:
- Demonstrated the feasibility of modified red blood cells to eliminate a model pathogen (bacteriophage) from the circulation. In an animal model, more than 99.9% clearance of circulating virus was achieved in less than 1 hour.
- Demonstrated feasibility of genetically engineering stem cells in vitro to express new gene products in order to develop modified stem cells to produce therapeutic products or provide automatic "booster" immunizations.
- Identified a synthetic SEB peptide capable of blocking binding of SEB to human MHC class II antigen.
- Demonstrated that monoclonal antibodies to TNF alpha and interferon gamma protect mice from lethal SEB challenge.

- Evaluated the role of antibodies in mice in mediating alphavirus vaccine interference. Found that non-neutralizing antibodies may act at the surface of infected cells to reduce the hose response to live alphavirus vaccines.
- Concluded a study on the efficacy in guinea pigs of anthrax vaccine against a trans of *B. anthracis* from numerous geographic areas.

Advanced Medical Diagnostics:

- Began studies to determine the feasibility of using exhaled nitric oxide (NO) as an early marker of infection of BW exposure.
- Developed an integrated sample preparation cartridge (to extract DNA from a biological sample) for connection to a miniature automated PCR apparatus.

Consequence Management Tools:

- ENCOMPASS (Enhanced Consequence Management Planning and Support System), an integrated set of consequence management tools, was developed and demonstrated with CBIRF (Marine Corps Chemical and Biological Incident Response Force). ENCOMPASS was used in Denver by CBIRF during the Summit of the Eight (June 1997) to provide plans, situational awareness and patient management in the event of a chemical or biological incident.

The following are accomplishments of medical biological defense research conducted by USAMRMC laboratories and/or their contractors.

Bacterial Agents

The countermeasures, technical barriers, and accomplishments in the biological threat category of bacterial agents are outlined below.

Countermeasures:

- Vaccines for immunity against threat agents.
- Antimicrobials for treatment of bacterial diseases.
- Forward deployed diagnostic systems.

Technical Barriers:

- Incomplete genetic information for all the threat agents.
- Lack of appropriate animal model systems for investigation of some bacterial threats and countermeasures.
- Limited capability to produce large bulk Good Manufacturing Practice (GMP) lots of vaccine candidates.
- Lack of suitable epidemiological situations in which to perform human clinical trials to evaluate efficacy of vaccines.
- Difficulty in field testing rapid identification kits under natural conditions.
- Difficulty in defining surrogate markers of protection.

Accomplishments:

- Found that all *B. mallei*/glanders strains were closely related antigenically.
- Determined antibiotic susceptibilities of *Burkholderia* (glanders) in mice and found that tetracycline was the most effective antibiotic, followed by ciprofloxacin and tobramycin.
- Demonstrated that killed *B. mallei* partially protected hamsters challenged with virulent organisms.
- Demonstrated two anti-spore activities of anti-PA antibodies-enhancement of phagocytosis by macrophages and inhibition of spore germination.
- Determined that serologic data suggest that endpoint ELISA titers do not correlate with predicting immunity to lethal plague challenge, but that other, more specific antibody subtypes may be useful as surrogate markers.
- Demonstrated that the assay for neutralization of anthrax toxin was useful in predicting the probability of survival in rabbits immunized with anthrax vaccine and challenged by the aerosol route.
- Completed sequencing of the GroES protein of *Rickettsia typhi*.
- Patent awarded for gene and protein applicable to the preparation of vaccines for *Rickettsia prowazekii* and *Rickettsia typhi* and the detection of both.
- Developed a nonhuman primate model for aerosolized Brucella, demonstrated that Rhesus monkeys develop bloodstream infection after aerosol challenge with as few as 100 colony forming units of *B. melitensis*.
- Improved a candidate Brucella vaccine strain of *purE*201 by eliminating its antibiotic resistance using gene replacement.
- Established an oral immunization regimen in mice using rough mutants of *B. melitensis*.
- Demonstrated that candidate vaccine strain, a mutant *purE*201, is cleared slowly from profoundly immunodeficient Rag-1 mice, indicating a role for nonspecific host defense in protection against this attenuated strain.
- Determined the DNA sequence of the *Yersinia enterocolitica* large virulence plasmid for comparison with similar plasmid found in *Y. pestis*. Determined that ribotyping is the best method for comparing strains.
- Initiated effort to isolate, characterize and detect ciprofloxacin-resistant *Y. pestis* mutants. Determined the wild-type sequence for genes known to be involved in resistance and characterized 20 resistant mutants.
- Evaluated numerous approaches to identify the enzymatic activities and targets of putative immunosuppressive effects of plague infection *in vivo*.
- Characterized inhibition of neutrophil migration as a potential biological activity of the V antigen of *Y. pestis*.
- Concluded a study on the efficacy in guinea pigs of anthrax vaccine against strains of *B. anthracis* from numerous geographical areas.

Protein Toxins

The countermeasures, technical barriers, and accomplishments in the biological threat category of toxins are outlined below.

Countermeasures:

- Antibodies (antitoxins) directed against common antigens of protein toxin molecules.
- Vaccines for immunity against protein toxin threat agents.
- Confirmatory assays to identify protein toxins specifically or classes of protein toxins.
- Drugs for supportive therapy of agent intoxication.
- Pharmaceuticals to delay or antagonize toxin effects.

Technical Barriers:

- Limited capability to produce large bulk GMP pilot lots of vaccine candidates.
- Lack of suitable epidemiological situations to perform human clinical trials to prove efficacy of vaccines and antitoxins.
- Difficulty in field testing diagnostic assays for toxins under natural conditions.
- Difficulty in producing polyvalent toxoid vaccines effective against classes of toxins.
- Lack of appropriate animal model systems for investigation of some protein toxin threats and countermeasures, or for testing treatment efficacy and safety in humans.
- Difficulty in defining surrogate markers of protection.

Accomplishments:

- Developed first pilot lot for expressing in yeast the C-fragment of BoNT/B made in compliance with the cGMP FDA regulations. This and other C-fragment candidates will be transitioned as potential vaccines.
- Completed the lot release and preclinical testing of the rBoNT/B(Hc) vaccine.
- Developed the fermentation and purification processes for the production of the rBoNT/A(Hc) vaccine.
- Obtained the first x-ray crystal structures for type A BoNT and for the C-fragment of tetanus toxin, a closely related clostridial neurotoxin.
- Determined spectroscopically that the secondary structure of BoNT/A & /B C-fragments in aqueous solution is predominantly composed of negative-strand elements, a result that is consistent with the x-ray diffraction data and with molecular modeling predictions. This information will be useful in the structural characterization of these candidate vaccine products.
- Developed and successfully tested a proof-of-concept DNA vaccine candidate for BoNT/A
- Modeled the secondary structural elements for BoNT/A-G.
- Successfully applied secondary structure and solvent accessibility predictive algorithms to the design of peptides for BoNT/A antibody response.
- Developed in-house the first sets of monoclonal antibodies that neutralize either BoNT/A or BoNT/B.
- Synthesized a potent polypeptide inhibitor for BoNT/A that will be used as a lead compound in future combinatorial chemistry syntheses.
- Screened a variety of thermolysin (the prototypic metalloprotease) inhibitors; effective concentration for the best compound was 20 μm against BoNT/B. This may become another lead inhibitory compound.
- Developed a novel *in vitro* system using biological membranes to examine the physiological activity of BoNT-induced ionic channels and their potential role as a target for chemotherapies to counteract the internalization of the toxin.

- Developed cell-free *in vitro* assays to study the actions of candidate metalloprotease inhibitors on the catalytic activity of botulinum toxin light chain. These systems monitor the rate of cleavage of the substrates synaptobrevin (serotype B) and SNAP-25 (serotype A) by capillary electrophoresis. Emphasis will be placed on greater assay automation and on faster separation of cleavage products.
- A primary mouse spinal cord culture system was examined for its suitability as a cellular model for botulinum toxin research. The cultures were found to be highly sensitive to serotypes A and B but relatively insensitive to serotype E, can be used to study the mechanisms of action of botulinum toxin, and to test therapeutic agents.
- Tested extensively the isolated mouse phrenic nerve hemidiaphragm preparation and found it to be highly suitable for evaluating botulinum toxin antagonists.
- Developed a synthetic approach and began the synthesis of a potential botulinum antidote.
- Maintained Chemical Repository so that putative drugs could be sent for testing against threats, *i.e.*, selected and sent 50 putative antibotulinum toxin agents to USAMRICD, for testing.
- Work on mechanisms of botulinum toxin A and on protectants performed completely *in vit*ro, thereby generating scientific progress without performing animal experiments.
- Described the use of a natural peptide, Buforin 1, to inhibit the toxic enzymatic activity of botulinum toxin B, making it a potential drug for counteracting botulinum toxicity.
- The SEB toxoid proteosome vaccine was found to be effective in protecting monkeys from SEB aerosol challenges (10-18 LD_{50}). A comparison study was conducted recently in monkeys for the efficacy of the new soluble SEB toxoid, the SEB toxoid-containing microspheres, and the SEB toxoid formulated with proteosomes.
- Chemically modified histidines of the SEB molecule and studied its biological activities. Used methods of genetic engineering to change the histidine codons of SEB genes by site-directed mutagenesis and cloned the mutated genes in *E. coli*. These SEB mutant proteins are under investigation for use as intranasal vaccines and as therapeutics.
- Completed ultrastructural studies to assess the effect of an incapacitating dose of SEB on Rhesus monkeys following an aerosol exposure. Demonstrated that the chosen SEB dose induced blastogenesis in 52%-57% of lymphocytes indicating high superantigenic activity of the toxin.
- Developed a primate test battery to assess behavioral incapacitation induced by nonlethal exposure to SEB as part of a collaborative effort with Division of Pathology [Walter Reed Army Institute of Research (WRAIR)] and Division of Toxinology (USAMRIID). This accomplishment earned 1998 Army Research and Development Awards for the two WRAIR scientists who directed this project.
- Extended the primate test battery to a touchscreen platform that greatly increases the flexibility and utility of the behavioral assessment capability.
- Demonstrated effectiveness of a newly developed, multi-channel telemetry device that assesses physiological parameters associated with SEB toxicity.
- Established a sublethal SEB exposure time course for dose-dependent production of eicosanoids, neuropeptides and cytokines.
- Identified three potential therapeutic agents against the toxic effects of SEB.
- Began cGMP vaccine recombinant SEB (rSEB) pilot lot, including seed stock,

fermentation, purification, formulation/vialing, QC testing and documentation, identity and rSEB/cGMP vaccine stability, safety, and reactogenicity.

- Standardized a potency assay for rSEB vaccine.
- Developed surrogate endpoint in animal models that will be used to evaluate the immune response in humans following vaccination for protection against the BW threat.
- Produced a lot of GMP SEB toxin to be used as a reference standard (Battelle/Centre for Applied Microbiology and Research, U.K.).
- Successfully completed bivalent (SEA/SEB) recombinant vaccine 12-month immunogenicity study in nonhuman primates.
- Initiated studies evaluating the effectiveness of SEB vaccines (both toxoid and rSEB) against lethality and incapacitation: nonhuman primate study of toxoid and rSEB vaccines.
- Correlated the structural features of SEB with its functional properties by designing and synthesizing 26 site-specific mutant proteins, sets of synthetic peptide fragments and truncated proteins.
- Identified several peptide fragments of the SEB molecule which in turn were developed into vaccine candidates that elicited neutralizing antisera production without associated effects of SEB toxicity.
- Initiated development of a transgenic mouse model of SE intoxication/incapacitation based on expression of higher affinity human receptor molecules.
- Developed a new and powerful computational method for the rapid prediction and assessment of protein-protein binding modes and their affinities for the genetically engineered mutants of the SEs.
- Predicted the binding characteristics of the rSEB and rSEA proteins with MHC class II molecules, and their oligomerization with T-cell receptors.
- Identified a synthetic SEB peptide capable of blocking binding of SEB to human MHC class II antigen.
- Demonstrated that monoclonal antibodies to TNF alpha and interferon gamma protect mice from a lethal SEB challenge.
- Demonstrated that chlorpromazine, an FDA-approved tranquilizer, protects mice from a lethal SEB challenge, and that pentoxifylline diminished the lethal effects of SE in mice.
- Demonstrated that SEB-induced production of mediators were centrally controlled by a battery of selected protein kinases and inhibitors of several kinase pathways blocked SEB's biological effects and abrogated SEB-induced lethality in a mouse model.
- Developed a potency assay for deglycosylated A-chain ricin vaccine and submitted a validation plan for this assay for approval.
- Determined that lyophilized deglycosylated A-chain ricin vaccine is chemically stable and maintains its potency for at least 18 months. (PerImmune, Inc.).
- Determined optimum vaccine schedule protecting mice and rats in a lethal aerosol challenge model for ricin.
- Conducted successful general and acute GLP safety testing on deglycosylated A-chain ricin vaccine (PerImmune Inc.).
- Produced a pilot lot of deglycosylated A-chain ricin vaccine in collaboration with an industrial partner (IntraCel).
- Determined high-dose and longevity safety parameters in a mouse model for the

deglycosylated A-chain ricin vaccine.
- Identified surrogate markers for immunological protection against aerosolized ricin.
- Determined the sequence-specific interactions of the ricin-rRNA binding determinant critical for the design of selective N-glycosidase inhibitors.
- Modeled *de novo* designed ricin inhibitors based on substrate analogs.
- Developed a synthetic method and commenced the synthesis of the ricin inhibitor.
- Tested *C. perfringens* iota toxin (a binary, lethal enterotoxin) and found that it does not elicit proinflammatory cytokines from human peripheral blood lymphocytes *in vitro*, unlike other bacterial enterotoxins (*i.e.*, staphylococcal enterotoxins [SE]) that represent BW threats and are very active at inducing a lethal cytokine cascade.
- Began receptor binding studies for iota toxin on various tissue culture cell lines and additional studies are ongoing to characterize the surface receptor on susceptible African green monkey kidney cells.
- Conducted aerosol challenge in rats using spores of *C. perfringens* and determined that this animal species is not susceptible to infection/intoxination by this route.

Viral Agents

The countermeasures, technical barriers, and accomplishments in the biological threat category of viral agents are outlined below.

Countermeasures:
- Vaccines for immunity against viral threat agents.
- Antibodies and antiviral drugs for treatment of viral disease.
- Devices and technologies for diagnosis of viral disease.

Technical Barriers:
- Lack of appropriate animal model systems for investigation of viral threats and countermeasures.
- Limited capability to produce large bulk GMP pilot lots of vaccine candidates
- Lack of suitable epidemiological situations in which to perform human clinical trials to evaluate efficacy of vaccines.
- Need for production of multivalent vaccines against heterologous viral agents.
- Difficulty in optimizing and comparing different expression vectors for recombinant products (vaccines and antibodies).
- Need for rapid virus identification technology.
- Difficulty in defining of surrogate markers of protection.

Accomplishments:
- Demonstrated that fibroblastic reticulum cells of lymphoid tissue are the early target cells of Ebola virus.
- Discovered that nonlethal infection of Ebola virus confers protective immunity to intraperitoneal (IP) challenge by a subsequent lethal dose of virus.
- Identified a reverse genetic system for Ebola virus in which virus is rescued from a clone copy of the viral genome. This allows manipulation of the genome to create attenuated

viruses for purposes of vaccination.

- Protected a mouse model against filovirus challenge using replicon Ebola virus RNA expressed in the VEE replicon system.
- Demonstrated protection against Marburg virus challenge in guinea pigs immunized using Marburg DNA cloned into a plasmid and delivered using a "gene gun".
- Demonstrated the first successful protection of non-human primates from lethal Marburg virus challenge after immunization with a genetically constructed replicon Marburg virus vaccine.
- Refined animal models of filovirus infection using conjunctival, oral, and aerosol routes of challenge.
- Discovered that a key pathogenic event in Ebola virus infection is destruction of mononuclear phagocytes.
- Discovered that serum from mice immunized against an adapted Ebola virus passively protects naïve mice from lethal disease.
- Identified candidate prophylactic agents for filoviruses, a group of hydrolase inhibitors, to be evaluated in a nonhuman primate challenge model.
- Designed and characterized novel monoclonal antibody complexes, targeting bound Marburg virus, for hepatic immune system clearance.
- Initiated development of an *in vitro* model system of Ebola virus replication that can be used to better understand early stages of virus infection as well as to investigate potential therapeutic compounds.
- Synthesized the enantiomers of 9-(trans-2′,trans-3′-dihydrocyclopent-4-yl)-3-deazadenine for evaluation as potential chemotherapeutic agents against Ebola and Marburg viruses.
- Established purity, stability, and reversion database for the deletion-mutant VEE vaccine candidate, V3526.
- Evaluated three VEE virus vaccines in laboratory mice. Three candidates, formalin-inactivated C-84, live-attenuated TC-83, and deletion-mutant V3526, were evaluated for efficacy and onset and duration of immunity. The V3526 was shown to be more attenuated and more immunogenic in protecting nonhuman primates than TC-83.
- Completed histopathological evaluation of CNS tissue of VEE exposed, susceptible and immunized mice. CNS invasion by VEE challenge is prevented in mice vaccinated with TC-83 or V3526. The deletion mutant V3526 showed significantly less neurovirulence in mice than the TC-83 preparation.
- Demonstrated inability of deletion-mutant V3526 to revert to wild-type VEE in the natural mosquito vector.
- Applied deletion-mutant technology to formulate WEE and EEE vaccine candidates. For WEE the best candidate, WE2102, elicited high serum neutralizing antibody titers in mice, reduced mortality, but did not affect morbidity.
- Generated a live-attenuated molecular clone of VEE subtype IE that appeared to be nonvirulent, immunogenic, and protective in animal models. It may serve as the basis for further development of a vaccine candidate.
- Evaluated the role of antibodies in mice in mediating alphavirus vaccine interference. Found that non-neutralizing antibodies may act at the surface of infected cells to reduce the host response to live alphavirus vaccines.

- Determined in animal models that deletion-mutant V3526 VEE vaccine candidate was less susceptible to interference by pre-existing alphavirus antibodies than the existing TC-83 vaccine.
- Developed a monkeypox model using nonhuman primates to evaluate efficacy of both the licensed and cell-culture-derived replacement vaccines against variola. Both vaccines showed protection against high dose aerosol challenge of monkeypox.
- Demonstrated ability to clone vaccinia glycoprotein genes into an alphavirus (VEE) replicon vector to assess this approach to a genetically engineered smallpox vaccinia.
- Mice immunized with L1R immunogen derived from vaccinia virus and delivered using a "gene gun" were completely protected against a lethal dose challenge of vaccinia.
- Demonstrated efficacy of DNA-polymerase inhibitors against variola virus.
- Demonstrated that monoclonal antibodies specific to L1R of vaccinia virus neutralized the virus in cell culture.
- Conducted histopathologic examination of monkeypox aerosol-challenged nonhuman primates, documenting fibrinonecrotic bronchopneumonia and diffuse dispersal of antigen in airway epithelium and surrounding interstitium.

Diagnostic Assays for Biological Warfare Threat Agents

The accomplishments in the diagnostic assays for biological warfare threat agents are outlined below. The objective of this effort is to develop the capability to confirm in biological samples the initial field diagnosis of a biological warfare threat agent.

Technical Barriers:

- Difficulty in field testing rapid identification kits under natural conditions.
- Lack of rapid confirmatory assays with "gold standard" sensitivity and specificity.
- Limited rapid deployable identification technology.

Accomplishments:

- Evaluated two molecular diagnostic approaches to identify pathogenic orthopoxviruses. These two assays will allow delineation between strains of orthopoxviruses including those that may have been genetically manipulated.
- Developed a specific and sensitive ELISA for *C. perfringens* alpha toxin, a lethal protein produced by all *C. perfringens* strains and intimately linked to the pathogenesis of this microorganism.
- Designed primers for cloning putative genes involved in regulation of iron binding proteins (*tonB*), murein biosynthesis (*murE*), methionine biosynthesis (*metL*, *thrA*), the chaperonins involved in protein translocation to the periplasm (*secE*), and transcription termination (*nusG*).
- Developed strategies and techniques to analyze lymphoid cells exposed (*in vitro* or *in vivo*) to biological or chemical threat agents to catalogue unique patterns of alterations in gene expression to use as surrogate markers of exposure to specific BW agents and predict patterns of impending illness.
- Developed monoclonal antibodies specific for Q fever (phases 1 and 2) to replace existing polyclonal antibodies in existing antigen capture ELISA.

- Developed improved monoclonal antibodies to VC 01 serotypes and developed antigen capture ELISA with improved sensitivity and specificity.
- Developed a monoclonal antibody to *Burkholderia mallei* to replace existing polyclonal antibodies in antigen capture ELISA.
- Developed methods for subtyping *Bacillus anthracis*.
- Developed and optimized methods for the isolation of viral RNA from environmental and clinical specimens.
- Developed an antigen capture ELISA for poxviruses.
- Developed a monoclonal antibody specific for botulinum toxin B to be used in the development of an antigen capture ELISA.
- Developed rapid PCR system for the detection of BW agents to be incorporated into a field deployable laboratory. Successfully field-tested this system for the detection of bacterial agents in aerosol collections.
- Transferred immunochromatographic hand-held assay technology to a selected commercial company for production quantity in service of the Joint Program Office for Biological Defense. Developed a prototype hand-held assay housing for its portability.
- Developed SEB, Ricin, *B. anthracis*, botulinum toxins A and B, and *F. tularensis* detection assays using the Bidiffractive Gating Biosensor.
- Constructed database of known DNA sequences relating to organisms of biological warfare concern. Currently includes over 4,800 genes. Continuing to add new DNA sequences and other capabilities to the database.
- Developed rapid, sensitive and specific immunochromatographic hand-held assays for SEA, SEC, Q fever, and *Y. pestis* non-F1.
- Developed rapid, single step PCR assays for the following agents: *B. anthracis*, *Y. pestis*, *Vibrio cholerae*, *Clostridium botulinum* A, *Clostridium botulinum* B, orthopox virus, and Venezuelan equine encephalitis (VEE) virus. These rapid PCR assays use fluorescent biosensor detectors capable of detection of BW agents in less than 25 minutes.
- Identified in collaboration with Lawrence Berkeley National Laboratory a new, chromosomal DNA marker for the identification of *B. anthracis* and developed PCR assays using that marker.
- Developed rapid, sensitive and specific immunochromatographic hand-held assays for VEE, pox, and Ebola viruses.
- Developed recombinant antibodies to ricin and botulinum toxin E that are being incorporated into diagnostic assays.

D.2.3 Advanced Development Accomplishments

The Joint Program Office for Biological Defense (JPO-BD) is a DoD chartered agency to provide intensive centralized management of medical and non-medical programs to expedite materiel solutions for validated biological defense deficiencies. Vaccine products will be further developed by the Joint Vaccine Acquisition Program (JVAP) under the auspices of the JPO-BD. Medical devices, diagnostics, and therapeutics will continue to be developed by USAMMDA. Vaccines directed against high threat agents will be produced and stockpiled to fulfill a 1.2 million Troop Equivalent Doses (TEDs) requirement [TED = the amount of vaccine required to

immunize a service member to protect against a biological warfare agent] . Vaccines against low threat agents will be produced to fulfill a 300,000 TEDs requirement.

The following products have transitioned from Tech base R&D to advanced development and are managed and funded by JPO-BD.

D.2.3.1 Botulism Immune Globulin (Human), Pentavalent (IND #1332)

- The IND remains open to accommodate emergency treatment requirements for exposure or possible exposure to botulinum toxin types A, B, C, D, or E.

D.2.3.2 Botulinum Type F Toxoid Vaccine (IND #5077)

- Completed the Phase 2 Safety and Immunogenicity clinical study of Botulinum Type F Toxoid Vaccine. The purpose of this study is to identify a vaccination schedule and route of vaccination that is safe and maximally immunogenic.
- The 12-month serology after the primary three-inoculation series of vaccinations has was drawn from the last cohort in the Phase 2 study and demonstrated that the immunogenicity of the purified Botulinum Type F Toxoid.
- The 1-year booster phase of the Phase 2 study is complete and 142 sera were above 0.25 IU/ml of antibody demonstrating the effectiveness of this vaccine.
- Provided product for a laboratory comparison of F toxoid with recombinant Fc product in animal efficacy experiments.

D.2.3.3 Anthrax Vaccine Human Adsorbed

- The sale of Michigan Biologic Products Institute (MBPI) by the state of Michigan was finalized. MBPI was purchased by BioPort that consists of the management team from MBPI and outside capital; it is a private sector entity without state of Michigan affiliation.
- Managed and funded efforts leading to the submission of a PLA amendment to the FDA for Anthrax Vaccine Adsorbed. The data was submitted to reduce the current schedule of six doses to a five-dose schedule that will provide protection against aerosol exposure to anthrax.
- Managed the anthrax vaccine production and stockpile to ensure sufficient vaccine is available to support the Secretary of Defense's anthrax immunization efforts.
- DoD continued to provide technical assistance to MBPI/BioPort to identify and correct FDA compliance deviations.
- Funded and provided oversight of production facility upgrades and ancillary support function renovation at BioPort that are critical to maintaining anthrax vaccine availability

D.2.3.4 Botulinum (Pentavalent) Toxoid Adsorbed (ABCDE) (IND#3703)

- Indemnification was granted for the conduct of the pivotal clinical trial for product approval.

- Protocol written and approved for pivotal clinical study. This protocol was briefed to the FDA April 1998 and accepted after coordination.
- Animal studies were completed demonstrating the equivalence of intramuscular (IM) and IP administration of toxin challenge doses. This study validated the use of the IP route of administration.
- Final reports were submitted to the FDA documenting (1) the validation of assays and the passive transfer of human antibody to an animal model (*i.e.*, guinea pig) in support of the Pentavalent Botulinum Toxoid vaccine licensure.
- A study demonstrating the effectiveness of human toxin neutralizing antibodies as a surrogate correlate of efficacy/protection against aerosol challenge with botulinum toxin was successfully completed.
- A botulism IM challenge study demonstrating the protective efficacy of human neutralizing antibodies transferred to guinea pigs was completed. This study will provide the data for the protective geometric mean titers for each of the botulinum serotypes.

D.2.3.5 Botulism Immune Globulin F(ab′)$_2$, Heptavalent, Equine, Types A, B, C, D, E, F, & G IND (#7451)

- Contracted for continued stability testing of the product.
- Filed IND with the FDA.
- Initiated Phase 1 Safety and Pharmacokinetics clinical study.
- Provided Botulinum Antitoxin Standards to Battelle Medical Research and Evaluation Facility used for the development of the Pentavalent Botulinum Toxoid (ABCDE).
- Manufactured 4,913 doses of cGMP Botulism Immune Globulin.

D.2.3.6 Botulism Immune Globulin (Human), Pentavalent (IND #1332)

- Conducted storage stability testing on this IND product.
- The IND remains open to accommodate emergency treatment requirements for exposure or possible exposure to botulinum toxin types A, B, C, D, or E.

D.2.3.7 Botulinum Type F Toxoid Vaccine (IND #5077)

- Completed the Phase 2 Safety and Immunogenicity clinical study of Botulinum Type F Toxoid Vaccine. The purpose of this study is to identify a vaccination schedule for the vaccine that is safe and maximally immunogenic.
- Provided product for a laboratory comparison of the F toxoid with a recombinant Fc product in animal efficacy experiments.

D.2.4 Joint Vaccine Acquisition Program (JVAP) Accomplishments

D.2.4.1 Prime Systems Contract

- The JVAP was initiated to consolidate all required manufacturing, testing, human clinical trial, logistical and regulatory expertise necessary to develop and license vaccines to protect against validated biological warfare agent threats.
- The JVAP prime system contract was awarded to DynPort Limited Liability Corporation (LLC) on 7-Nov-97. The basic contract consists of the storage, distribution and testing of the DoD contingency stockpile of Biological Defense (BD) vaccines and the development and licensure of 3 BD vaccines: Q-fever vaccine, Tularemia vaccine, and Vaccinia Virus vaccine. The contract has options for the development and licensure of an additional 15 BD vaccines. These options will be exercised as promising vaccine candidates transition into advanced development.
- Began work 2-Mar-98 after GAO resolution of contract award protest.
- Continued advanced development of these BD vaccine candidates through DynPort's use of government laboratories and facilities as DynPort's application for indemnification of unusually hazardous risks was being processed.
- DynPort is participating with government tech-base to help vaccine candidates transition into advanced development faster with reduced risk.
- Coordinated and conducted a meeting with the FDA to update Center for Biologics Evaluation and Review staff on the JVAP, to introduce the JVAP-Project Management Office and their Prime Systems Contractor (DynPort) and to describe the current vaccines included in the program.

D.2.4.2 Contingency Stockpile of Biological Defense (BD) Vaccines

- Transfer of the contingency stockpile of Biological Defense vaccines from the Salk Institute Biologics Development Center to McKesson BioServices was completed. McKesson BioServices is the DynPort sub-contractor for vaccine storage and distribution. McKesson BioServices has a state of the art facility dedicated to the storage of the BD investigational new drug (IND) contingency stockpile. The facility features redundant security systems, fully automated temperature monitoring, back up power system that ensures fully automatic transfer to a natural gas generator, and the capacity to meet the current and projected stockpile storage requirements.

D.2.4.3 Advanced Development of the Tularemia Vaccine

- Reviewed historical records and identified technical and regulatory issues to form the basis for a scientifically sound, feasible plan for the advanced development of a live attenuated tularemia vaccine.
- Selected a National Drug Company vaccine candidate as parent seed for development of the new vaccine for tularemia.
- Initiated process definition studies at the Life Science Division, Dugway Proving Ground to characterize large scale manufacturing procedures for the new tularemia vaccine.

D.2.4.4 Advanced Development of the Q-fever Vaccine

- Reviewed historical records and identified technical and regulatory issues to form the basis for a scientifically sound, feasible plan for the advanced development of a Q-fever vaccine.
- Met with a potential manufacturing subcontractor to discuss how their product meets our user's requirement.
- JVAP has received concurrence from our user about the suitability of this vaccine candidate.

D.2.4.5 Advanced Development of the Smallpox Virus Vaccine (Vaccinia Virus)

- Reviewed historical records and identified technical and regulatory issues to form the basis for a scientifically sound, feasible plan for the advanced development of a cell culture vaccinia vaccine for smallpox.
- Prepared a clinical protocol to evaluate the candidate vaccines administered by scarification.
- Guided protocol through all internal review boards and FDA review.
- Initiated process definition studies to evaluate large-scale production methods.
- Began discussions with the Department of Health and Human Services about the feasibility of scale-up production for the DoD vaccine to obtain for a civilian stockpile.
- Clinical protocol has stalled due to regulatory concerns about Vaccinia Immune Globulin, which is required before immunizations can take place.
- Baxter, current license holder for VIG, no longer plans to manufacture this product. JVAP market survey information from potential manufactures is being forwarded to DynPort to manage a new manufacturing and licensure effort for this product.

D.2.4.6 International Cooperative Research and Development

- The JVAP-Project Management Office conducted technical discussions with representatives of the United Kingdom and Canada about cooperative research and development agreements for Biological Defense vaccine products. A conceptual approach to tri-national cooperative research and development has been developed and is under review by the JPO-BD.
- Proposed recombinant plague vaccine candidates from the U.S. and United Kingdom recently underwent a pre-IND review at the FDA. This collaborative approach between the two countries leverages tech-base and advanced development efforts to provide a safe and effective vaccine protecting against aerosol exposure to *Yersinia pestis*.

D.3 MEDICAL NUCLEAR (RADIOLOGICAL) DEFENSE RESEARCH PROGRAM

D.3.1 Fielded Products

Advances in medical R&D significantly impact the warfighting mission by sustaining unit effectiveness through conserving the fighting strength of our service members. The individual service member whose performance is decremented by illness is significantly more likely to become a traumatic casualty. In this era of small, but highly lethal forces, loss of only a few team members can dramatically diminish a unit's capability. Medical R&D products (materiel and non-materiel solutions) provide the foundation that ensures the fielding of a flexible, sustainable, modernized force across the spectrum of conflict and in the full breadth and depth of the battlefield. Overcoming medical threats and extending human performance have provided a significant increase in military effectiveness in the past and present the potential for future enhancement on military operational effectiveness. Some of the fielded materiel and non-materiel solutions by medical radiological defense R&D are:

- Cytokine-based therapeutic applications to prevent the two major fatal syndromes—sepsis and uncontrolled bleeding—following acute radiation injury.
- Cytogenetic biodosimetry service operating to measure individual radiation exposure using blood samples.
- NATO Handbook on the Medical Aspects of NBC Defensive Operations, Volume 1-Nuclear (AMedP-6).
- Medical Effects of Ionizing Radiation (MEIR) Course—Training for approximately 660 Medical Department personnel in FY98.
- Videotapes and CD-ROM of MEIR course lectures produced for distribution to military medical units.

D.3.2 Nuclear Defense Research and Development Accomplishments

The nuclear (or radiological) defense research and development technical barriers and accomplishments during FY98 are grouped in the following threat categories:

- Prompt high-dose radiation.
- Protracted low-dose radiation.
- Combined radiation and chemical or biological agents.
- Embedded Depleted Uranium.

"*Prompt high-dose radiation*" refers to the deposition of high-energy radiation in biological tissues in very short periods of time. Sources of high-energy radiation include emissions within the first 60 seconds of a nuclear weapon detonation and "criticality events" that occur when a nuclear reactor achieves peak energy output either accidentally or through an intentional act. The high linear-energy-transfer imparted by the neutrons of these sources causes significant

tissue injury within seconds of exposure, resulting in both short- and long-term health consequences.

"*Protracted low-dose radiation*" refers to the deposition of low-energy radiation in biological tissues over extended periods of time. Sources of low-energy radiation include fallout from nuclear weapon detonations, radiological dissemination devices, and any other source of environmental radiation contamination. Health consequences are generally intermediate- to long-term and result from cumulative tissue injury accruing over time due to chronic exposure. Health consequences can be exacerbated further when radionuclides are deposited internally by ingestion, inhalation or through open wounds in the external integument.

"*Combined radiation and chemical or biological agents*" refers to the amplified health consequences when chemical or biological insults are incurred in conjunction with radiological injury. Both clinical and subclinical exposures to ionizing radiation compromise host defenses against a variety other stressors, including infectious agents and chemical toxicants. Exposures to doses of radiation and infectious or chemical agents that are by themselves sublethal can produce mortality rates of nearly 100% when combined.

"*Embedded Depleted Uranium"* refers to the metal used in penetration munitions and armor and the resultant radiological and toxicological consequences to personnel injured by embedded fragments of these munitions. Because of the unique and poorly understood radiological and toxicological properties of embedded depleted uranium, knowledge of the immediate and long-term risks is limited. Current treatment strategies are not well developed for personnel with tissue embedded depleted uranium and conventional diagnostic capabilities make it difficult to ascertain that personnel are injured with embedded depleted uranium.

The Medical Radiological Defense Research Program focuses on developing medical countermeasures to the health consequences of both prompt high-dose and protracted low-dose exposures to ionizing radiation. It also develops experimental data detailing combined NBC medical effects needed by computer modeling programs for casualty prediction. Specific research on medical countermeasures includes work on prophylactic and therapeutic drugs, drug delivery devices to enhance efficacy and simplify administration under field conditions, and combined prophylactic/therapeutic protocols to further enhance efficacy. Work also focuses on developing novel biological dosimetry techniques to measure individual absorbed doses. Knowledge of the dose of radiation absorbed helps guide medical treatment decisions and saves lives. It also provides field commanders with an assessment of the radiological health of deployed forces and leads to better-informed operational decision making.

Threat Category: Prompt High Dose Radiation

The countermeasures, technical barriers, and accomplishments in the threat area of prompt high dose radiation are outlined below.

Countermeasures:

- Advanced medical treatment strategies for radiation injuries.

- Drugs designed to increase resistance of soldiers to radiation and protect the soldier against radiation injury without compromising performance.
- Drugs designed to prevent the onset of radiation-induced performance decrements such as fatigue, nausea and vomiting.
- Biological dosimetry techniques for rapid injury assessment needed to guide medical treatment decisions and assessment of radiological health of combat units.

Technical Barriers:

- Need for reduction of the performance-degrading toxicity of prophylactic drugs that otherwise have good efficacy for the prevention of radiological injury.
- Need to advance knowledge of cellular, sub-cellular and molecular mechanisms of radiological injury to improve rational development of prophylactic and therapeutic drugs.
- Need for extending the stability of a prophylactic drug to allow its use in a slow-release delivery device for extended bioavailability and enhanced efficacy.
- Difficulty in identifying and calibrating biological markers that can both indicate the amount of absorbed radiation dose and differentiate whole-body from partial-body exposure.
- Inability to automate sample preparation and reducing sample preparation times of cytogenetic biodosimetry tests.

Accomplishments:

- Completed pilot demonstration of improved clinical support protocol (modified antibiotic and platelet transfusion regimens) for acute, potentially fatal radiation injury.
- Continued assessment and optimization of a combined radioprotectant, cytokine, and clinical support treatment modalities for enhancing survival following acute, lethal irradiation.
- Developed new prophylactic strategy for reducing acute radiation injury based on (a) apoptotic and reproductive mechanisms-based tissue injury and pathology, (b) low-toxicity drug selection, (c) pharmacologic quenching to further reduce toxic side effects, and (d) new drug delivery alternatives.
- Simplified sample preparation procedure used in cytogenetic assays to assess biologically absorbed radiation dose.
- Completed initial studies extending the application of radiation dose measuring protocols to exposure scenarios involving incremental doses of gamma and fission neutrons.

Threat Category: Protracted Low Dose Radiation

The countermeasures, technical barriers, and accomplishments in the threat area of protracted low dose radiation from nuclear fallout, radiological explosive devices, etc., are outlined below.

Countermeasures:

- Advanced medical treatment strategies for protracted radiation to mitigate injuries from both external and internal sources of radioactivity.

- Drugs designed to protect personnel from the early and late effects of ionizing radiation without compromising performance pharmacologic intervention strategies that protect against both early and late health effects arising from cellular and molecular damage caused by ionizing radiation.
- Improved techniques to detect and remove internally deposited sources of radioactivity
- Improved drug delivery systems that provide non-encumbering protection during the entire period of radiation exposure.
- Enhanced biodosimetry technique that can differentiate prior from recent exposures to radiation.

Technical Barriers:

- Lack of suitable radiation sources to study the effects of chronic exposure at relevant doses.
- Difficulty in manipulating cellular repair mechanisms.
- Toxicity of chelating agents used to remove sources of radioactivity.
- Brief periods in which traditional radioprotective drugs are active.
- Toxicity of radioprotective drugs used over protracted periods of time. Limited knowledge of DNA damage surveillance and repair mechanisms under protracted exposure conditions hinders development of pharmacologic agents to prevent late-arising cancers.
- Need to reduce the toxicity of heavy metal chelating agents while maintaining their efficacy.
- Need to extend bioavailability of prophylactic drugs to achieve maximum long-term protection.
- Potential cumulative toxicity of prophylactic drugs (antimutagenic and anticarcinogenic agents) when used for extended periods.
- Lack of a sustained drug delivery system of radioprotectants.
- Microbial resistance to antibiotics.

Accomplishments:

- Developed new prophylactic strategy for reducing chronic radiation injury based on (a) improved understanding of tissue damage and repair and subsequent late-arising disease, (b) selection of low-toxicity drugs that enhance tissue repair and minimize gene mutations, and (c) new slow release drug delivery systems that extend the radioprotective window.
- Established therapeutic drug assay to monitor blood levels of prophylactic drugs in support of studies to develop sustained drug delivery systems.
- Demonstrated use of implanted capsules as possible approach to provide sustained efficacious delivery of prophylactic drugs.
- Developed novel protocols using a fluorometric PCR for precise quantifiable measurements of molecular responses to radiation (oncogene expression, mitochondria DNA deletions) that can provide advanced biological markers for radiation dose assessments.
- Observed that ionizing radiation induces a specific deletion in mitochondrial DNA and alterations in oncogene mRNA expression, both of which appear to occur in dose-

dependent fashions.

Threat Category: Combined Radiation and Chemical or Biological Agents

The countermeasures, technical barriers, and accomplishments in the threat area of combined effects of nuclear ionizing radiation with trauma, burns, infection, or chemical toxicants radiation and trauma, burns, and infection are outlined below.

Countermeasures:

- Radiotherapeutic agents designed to decrease morbidity and mortality from multi-organ system failure due to the combined effects of radiation, trauma, burns, and infection or chemical toxicants.
- Radioprotective drugs designed to harden the soldier against the effects of radiation in combination with trauma, burns, infection, or chemical toxicants.
- Combined therapeutic agents designed to decrease morbidity and mortality from combined exposures and to enhance innate immune responses.
- Computer models for predicting casualties following combined exposure to low levels of ionizing radiation and biological warfare/chemical warfare agent aerosols.

Technical Barriers:

- No surrogate models for extrapolating data to humans.
- Limited animal models that are optimum for both radiation and a biological warfare or chemical warfare agent.
- Need to gain access to radiation sources and biological containment facilities in order to complete full range of experiments on combined effects of radiation and BW agents.
- Growing number of microbial organisms resistant to antibiotics.
- Accounting for variability in sensitivities of biological systems to different radiation qualities (*e.g.*, neutron *vs.* gamma radiation).
- Mechanism of action of cell-growth factors is not well understood.
- Sensitivity of bone marrow progenitor cells to low doses of ionizing radiation.

Accomplishments:

- Quantified increased mortality rates in irradiated mice infected via pulmonary route with *Bacillus anthracis* (Sterne) spores.
- Initiated studies to assess effects of radiation on immune status after vaccination with anthrax vaccine.
- Established in vitro and in vivo model systems to assess radiation/viral interactions.
- Established capability to integrate health consequences of radiation/biological warfare agent interactions, extrapolated from animal model studies, into the Consequence Assessment Tool Set (CATS).
- Identified synergistic consequence of combined exposure to sublethal radiation and therapeutic levels of PB resulting in redistribution of blood flow.
- Developed enhanced treatments for radiation-associated infections using immune system stimulators.

Threat Category: Embedded Depleted Uranium

The countermeasures, technical barriers, and accomplishments in the threat area of embedded depleted uranium are outlined below.

Countermeasures:

- Rapid assessment clinical analyses to identify personnel wounded with embedded depleted uranium.
- Safe and effective treatment strategies to minimize long-term health risks.

Technical Barriers:

- Determining the redistribution and toxicological consequences of exposure to embedded fragments of depleted uranium.
- Developing the reagents needed to improve sensitivity of tests to detect uranium.
- Developing or modifying pharmacological treatments to increase efficacy and reduce toxicity.

Accomplishments:

- Determined from studies designed to simulate embedded depleted uranium that depleted uranium from embedded fragments distributes to tissues far from the site of implantation.
- Described preliminary findings of carcinogenicity, neurotoxicity, and immunotoxicity of embedded depleted uranium fragments.
- Developed a new method for the colorimetric measurement of urinary uranium concentration.
- Developed a new method for the identification of uranium fragments in wounds.

D.3.3 Predevelopment Products

Technical developments in predevelopment products for medical radiological defense include the following:

- *Iloprost/Misoprostol/3D-MPL/WR-3689*
- "Slow release" radioprotectant for longer protection time for individuals at risk of high, potentially lethal levels of ionizing radiation.
- Nontoxic immune system stimulator for protection against radiation-induced immunosuppression and associated infection.
- CATS model enhancements to incorporate radiation/BW interactions.
- Product improvement of the cytogenetic biodosimetry system by automation of satellite scoring subsystem to increase sample throughput.
- Rapid and sensitive method to measure urinary uranium concentration.

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